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Fungal pathogens of 'cat's claws' from Brazil for biocontrol of *Macfadyena unguis-cati*

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Abstract—Recent searches in Brazil for plant pathogens with potential for use as classical biocontrol agents of *Macfadyena unguis-cati* yielded five fungal species, two previously undescribed. These are described herein and preliminary evaluations based on field and laboratory observations of the diseases with which they are associated are given. These are: *Guignardia mangiferae* (leaf spots), *Meliola herteri* (black mildew), *Passalora macfadyenae* sp. nov. (leaf spots), *Pseudocercospora unguis-cati* (leaf spots) and *Prospodium macfadyenae* sp. nov. (rust). The two newly described species, *Pa. macfadyenae* and *Pr. macfadyenae*, are regarded as having the greatest potential for use in classical biological control.

Key words —cat's claws, new species, plant pathology, taxonomy

Introduction

Invasion of natural ecosystems by exotic species represents one of the biggest threats to biodiversity, as now acknowledged by scientists and governments throughout the world (IUCN 2000). Once an alien organism has invaded, the sole long-term sustainable management alternative available for mitigating it through human intervention is the introduction of natural enemies collected in the pest's centre of origin in a process known as classical biological control. *Macfadyena unguis-cati* (L.) A.H. Gentry (*Bignoniaceae*) — common name cat's claws (in Brazil: unha-de-gato, unha-de-morcego) — is a woody vine that has a broad distribution in its native range occurring from southern Brazil and Argentina to Mexico (Everett 1980). *Macfadyena unguis-cati* takes its popular names from the tendrils, which have acute divided ends that appear and function like claws, allowing the plant to firmly attach to trees or other supports on which it grows (Plate 1). At maturity it produces abundant showy yellow flowers making it a useful ornamental plant for cultivation on fences

and garden walls (Lorenzi & Souza 1995). Because of its value as an ornamental it was introduced to regions outside its native range, where it escaped from gardens to become a noxious invader of native forests, particularly in the USA, Australia, China, and South Africa (Henderson 1995, FLEPPC 2005, Williams et al. 2008, Osunkoya et al. 2009, Dhileepan et al. 2010). Its vigorous vine habit allows it to ascend tall trunks reaching the treetops (Plate 2) branching and spreading over top of the forest canopy. The combination of the biomass weight and shading of underlying vegetation can be very harmful and even cause the death of the largest plants in infested areas and suppress the development of smaller plants and seed germination of native plants (Neser 1996, Huang et al. 2009, King & Dhileepan 2009). In Florida, *M. unguis-cati* is listed as a Category 1 weed, a rank reserved for species capable of altering plant communities by substituting and even permanently changing the natural structure of an ecosystem and its ecological functions (FLEPPC 2005). Between 2003 and 2005 a search was conducted in native situations in Brazil for fungal pathogens attacking *M. unguis-cati* that might have potential as biocontrol agents. This publication presents the results of this preliminary survey with particular reference to fungal taxonomy and the potential to use mycobiota in classical biological control.

Material & methods

Prior to the field survey, herbarium records were completely searched for occurrence and distribution of *M. unguis-cati* in the following Brazilian herbaria: Herbário Fanerogâmico e Criptogâmico do Instituto Agronômico (IAC); Herbário Maria Eneyda P. K. Fidalgo (SP); Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RB); Herbário do Museu Nacional do Rio de Janeiro (R); Universidade Federal de Viçosa (VIC); Museu Botânico Municipal (MBM); and Herbário José Badini (OUPR). Using this information, we prepared an itinerary for surveying the southern and southeastern Brazilian states Minas Gerais, Rio de Janeiro, Espírito Santo, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul. Surveys were conducted between January 2003 and December 2005. Selected sites were visited and carefully scrutinized for the presence of *M. unguis-cati.* For details on the general survey and laboratory methodologies see Barreto & Evans (1994). Freshly collected samples were examined under a stereomicroscope. Slides containing free-hand sections of colonized tissues or fungal structures scraped from the surface of infected tissues were mounted in lactophenol or lactofuchsin. Isolations of non-biotrophic fungi were attempted by directly transferring spores or other fungal structures to plates containing Vegetable Broth Agar (VBA, Pereira et al. 2003), under a dissecting microscope with a sterilized fine point needle. Observations, measurements, photographs and line drawings were prepared using an Olympus BX 50 light microscope fitted with a camera (Olympus Evolt SC 50) and drawing tube.

For scanning electron microscopy the samples were fixed in 2.5% glutaraldehyde + sodium cacodylate buffer (0.1mol L^{-1}, pH 7.2) (1:1) for 1h at room temperature, washed six times in 0.1mol L^{-1} sodium cacodylate buffer (10 m each washing period), post-fixed

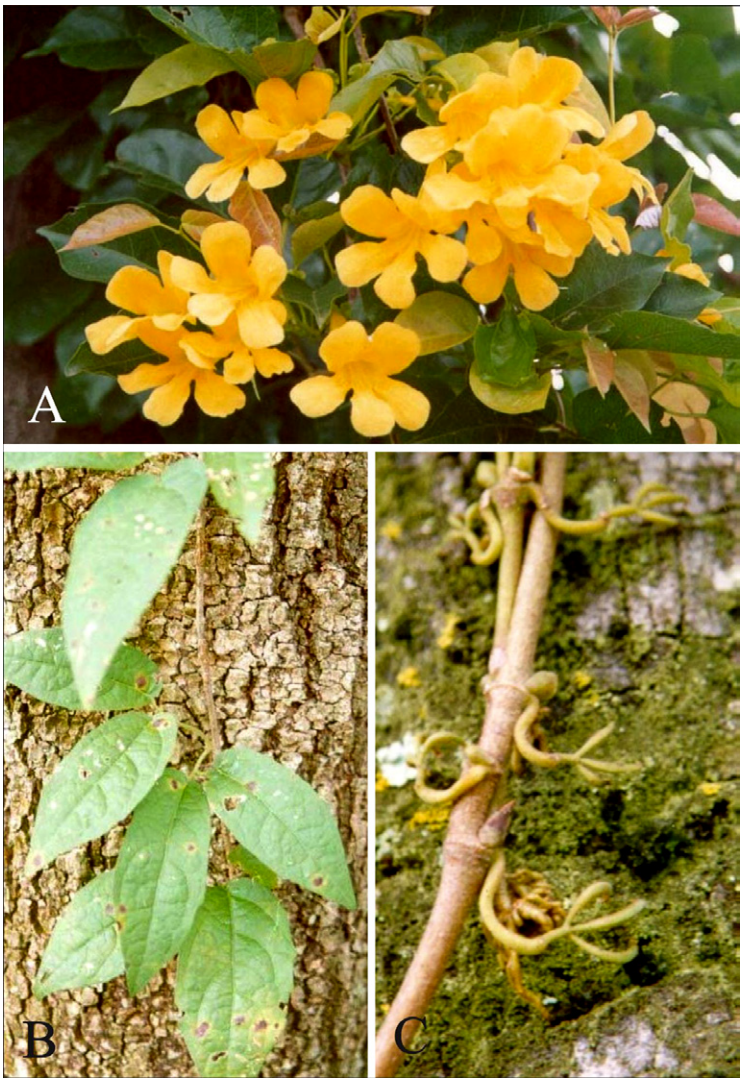

Plate 1. *Macfadyena unguis-cati*: a. typical large, yellow, trumpet-shaped flowers; b. climbing on tree trunks; c. clawed tendrils which enable plant to climb up against walls, tree trunks, and other supports.

by immersion in 1% O_sO_4 prepared in cacodylate buffer 0.1 mol L^{-1} (1:1), and kept for 4 h at 4-8 °C then washed again six times in buffer, dehydrated by 10-min long successive transfers in an alcohol solution (30, 50, 60, 70, 80, 95, 100%) gradation series at room temperature, dried in a critical point dryer (Baltec model 030) with CO_2 as transition

Plate 2. *Macfadyena unguis-cati* climbing trees in the field in Brazil.

fluid, and coated with 20 nm thick gold in a sputter coater (Balzers® model FDU 010) before examination in a Carl-Zeiss Model LEO VP 1430 electron microscope.

Representative specimens were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

Taxonomy

Numerous samples of diseased *M. unguis-cati* were collected during the survey. Five different fungal pathogens were recognized in association with cat's claws. Two fungal species were isolated in pure culture. The five fungal pathogens were associated with the following diseases: leaf spots (3), black mildew (1), and rust (1). Descriptions of the identified fungi are given below.

Guignardia mangiferae A.J. Roy, Indian Phytopath. 20: 348. 1968. PLATE 3

Lesions on living leaves, minute dark tar-spots corresponding to fungal conidiomata surrounded by faint chlorotic areas, occasionally associated to larger necrotic areas. External mycelium absent. Pseudothecia hypophyllous, immersed, solitary or in groups, subglobose, 136.5–113 µm, wall composed of texture angularis, 17.5–27.5 µm, dark brown to black, smooth. Asci bitunicate, subclavate to cylindrical, 46.5–82 × 11–14 µm, eight-spored. Ascospores biseriate, fusiform-elliptical, 12–14 × 4.5–5 µm, unicellular, guttulate, hyaline, smooth, surrounded by a thick mucilaginous coat.

MATERIAL EXAMINED: **BRAZIL. SÃO PAULO**, on *Macfadyena unguis-cati*, 16 April 2005, OLPereira (VIC 30492); **MINAS GERAIS**, on *Macfadyena unguis-cati*, 25 July 2011, MSilva & OLPereira (VIC 31759).

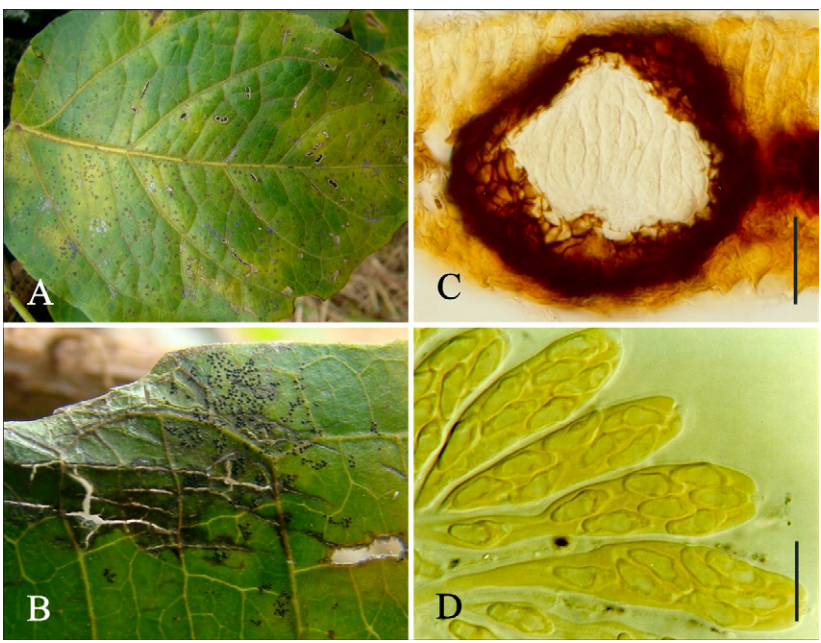

PLATE 3. A–B: *Guignardia mangiferae* on *Macfadyena unguis-cati*. A. Faint chlorotic symptoms on infected leaves; B. circular individual pseudothecia on adaxial leaf surface. C–D: *Guignardia mangiferae* (VIC 30492). C. Section through a pseudothecium showing the asci; D. bitunicate asci containing biseriate, hyaline ascospores. Bars: C = 30 µm; D = 10 µm.

Comments: *Guignardia mangiferae* was observed causing some damage to few isolated leaves on infected *M. unguis-cati* plants (Plate 3A–B) but is regarded here as having no potential for classical biocontrol of *M. unguis-cati*. This is the first record of *G. mangiferae,* a polyphagous opportunistic pathogen of numerous plant families (Rodrigues et al. 2004), on a representative of *Bignoniaceae* (*M. unguis-cati*) worldwide.

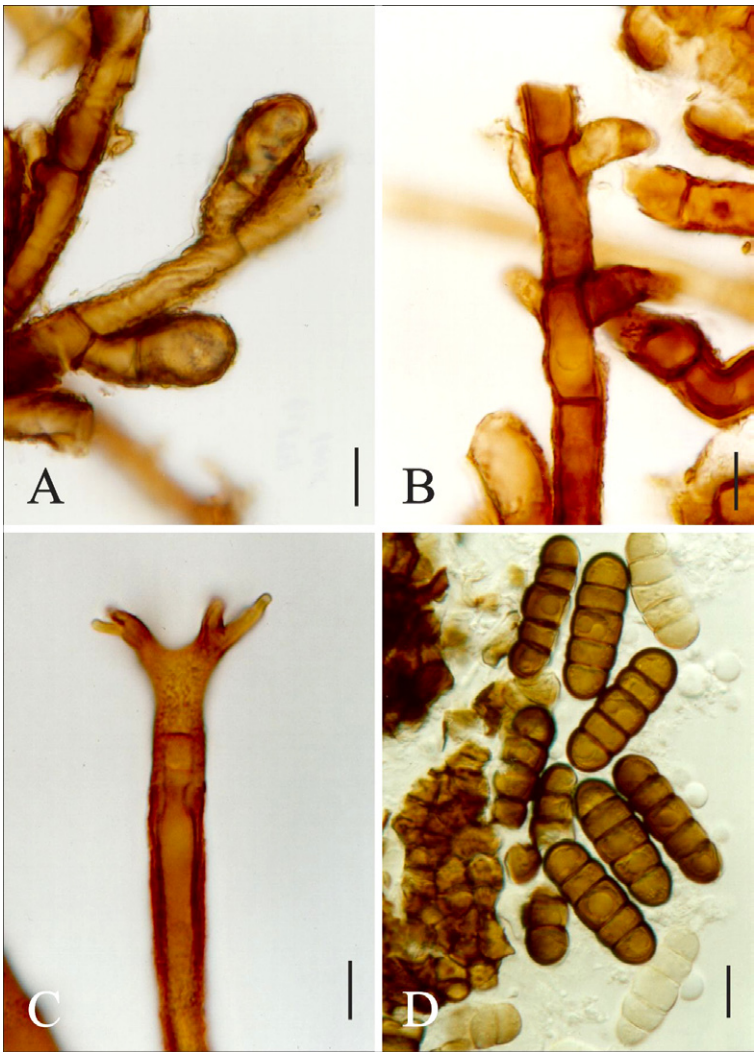

Plate 4. *Meliola herteri* (VIC 30485): A. external hyphae with alternate appressoria; B. opposite conidiogenous cells; C. branching-dentate setae; D. ascospores. Bars: A–C = 10 µm; D = 20 µm.

Meliola herteri Hansf., Sydowia 9: 65. 1955. PLATE 4

Colonies amphigenous, mostly epiphyllous, 4–50 mm diam, sooty, scattered, numerous, confluent, black. External mycelium net-forming, 6–9.5 µm diam hyphae, slightly undulate, alternately branching at acute angles, dark brown, septate, producing phialides (mucronate hyphopodia) and appressoria (capitate hyphopodia), smooth, brown; appressoria alternate apical, stalk-cells cylindrical 6–16 × 5.5–9.5 µm, head-cells oval or elliptical, 14–19 × 9.5–14 µm dark brown; phialides intermixed with appressoria, ampulliform to cylindrical, 14–25.5 × 5.5–9 opposite or alternate, scarce. Perithecia superficial, aggregate, globose, 112–155 µm diam. Mycelial setae scattered, straight to slightly curved, 155–253 × 6–9.5 µm, the apex usually 2–4 furcate and secondarily 2–3 branched, brown. Asci evanescent at maturity. Ascospores cylindric to oblong, 44–50.5 × 13–19 µm, constricted at the septa, apex and base rounded, 4-septate, brown, smooth.

MATERIAL EXAMINED: **BRAZIL. RIO GRANDE DO SUL**, on *Macfadyena unguis-cati*, 6 August 2004, OLP (Pereira) (VIC 30485).

COMMENTS: Three *Meliola* species are known to occur on *M. unguis-cati*: *Meliola thaxteri* Hansf., *M. bidentata* Cooke, and *M. herteri* (Hansford 1961). The morphology of the specimen collected on *M. unguis-cati* during the survey clearly placed this fungus within *M. herteri* as described by Hansford (1961). *Meliola herteri* has been previously recorded on *M. unguis-cati* in Paraguay, *Amphilophium vauthieri* DC. in Argentina, and *Parabignonia maximilianii* (Mart ex DC.) Bureau in Brazil (Hansford 1961). This is the first report of *M. herteri* on *M. unguis-cati* in Brazil. *Meliola herteri* is, as are the *Meliolaceae* in general, a weak obligate biotroph and the black mildew diseases the family causes do not significantly affect their hosts (Hansford 1961). This fungal group therefore has little relevance for use in biological control.

Passalora macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, **sp. nov.** PLATE 5

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Morphologically similar to *Passalora adenocalymmatis* and *Passalora pyrostegiae* but differs in shorter (31.5–114 µm) conidia, longer (16-114 µm) conidiophores, and a well-developed stroma.

TYPE: Brazil, Minas Gerais, on *Macfadyena unguis-cati*, 18 May 2004, OLP (Pereira) (VIC 30480, **holotype**).

ETYMOLOGY: from the host genus name.

Lesions on living leaves, similar to those cause by *P. unguis-cati* but somewhat darker centrally, 4–55 mm in diam., coalescing to cover large areas of leaf surface and leading to leaf blight. Internal mycelium indistinct. External mycelium absent. Stromata erumpent, 63–142 × 35–95 µm, dark brown. Conidiophores hypophyllous arising from stromata, in dense fascicles, cylindrical, straight to

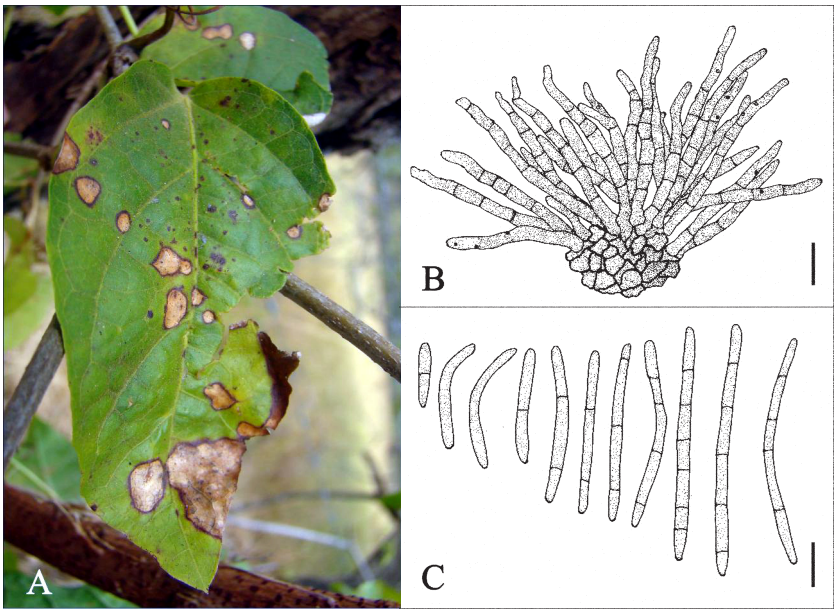

Plate 5. a: *Passalora macfadyenae* on *Macfadyena unguis-cati*: necrotic symptoms on infected leaves. b–c: *Passalora macfadyenae* (VIC 30480): b. conidiophore fascicle on stroma; c. conidia (straight to slightly curved). Bars = 10 µm.

slightly curved, 16–114 × 3–6 µm, 0–8 septate, unbranched, brown, smooth. Conidiogenous cells polyblastic, integrated, mostly cylindrical, 5–17.5 × 3.5–5 µm, brown. Conidiogenous loci conspicuous, 1–3 per conidiogenous cell, 1–2.5 µm, thickened and darkened. Conidia dry, solitary, cylindrical-obclavate, straight to slightly sinuous, 31.5–114 × 3–4.5 µm, base obconically truncate, apex rounded, 1–12 septate, hilum thickened and darkened, subhyaline to olivaceous, smooth.

Additional material examined: **BRAZIL**. **São Paulo**, on *Macfadyena unguis-cati*, 25 July 2004, OLPereira (VIC 30487).

Comments: No *Passalora* species are known to associate with *Macfadyena* taxa. Ten *Passalora* species are known to attack *Bignoniaceae* representatives, but all are dissimilar to the fungus collected on *M. unguis-cati*. Conidial sizes are useful in separating *P. macfadyenae* from many of the similar *Passalora* species of (Table 1). *Passalora tecomariae* Crous & B. Sutton and *P. tabebuiae-ochraceae* Inácio & Dianese differ from the new species in wider conidia, *P. tabebuiae* (J.J. Muchovej & F.A. Ferreira) U. Braun & Crous has narrower conidia, and *P. adenocalymmatis* U. Braun & Crous, *P. arrabidaeae* (Chupp & Viégas) Crous et al., and *P. pyrostegiae* (Viégas) U. Braun & Crous have longer conidia, smaller

Table 1. Conidial size of *Passalora* species recorded on members of the *Bignoniaceae*.

Species	Conidia (µm)	References
P. adenocalymmatis	35–150 × 3–4.5	Chupp (1954), as "*Cercospora adenocalymmae*"
P. arrabidaeae	35–150 × 3.5–5	Chupp (1954), as *Cercospora arrabidaeae*
P. catalpae	40–120 × 2.5–4.5	Chupp (1954), as *Cercospora catalpae*
P. catalparum	35–125 × 3.5–6	Chupp (1954), as *Cercospora catalparum*
P. leprosa	30–80 × 3.5–6	Chupp (1954), as *Cercospora leprosa*
P. macfadyenae	31.5–114 × 3–4.5	This publication
P. markhamiae	16–92.5 × 2.5–55.5	Liu & Guo (1982)
P. pyrostegiae	25–170 × 3–4.5	Chupp (1954), as *Cercospora pyrostegiae*
P. tabebuiae	(6–)8–30(–34) × 2.5–3	Muchovej & Ferreira (1981)
P. tabebuiae-ochraceae	31–75 × 5–8	Inácio & Dianese (2006)
P. tecomariae	35–90 × 5–6	Crous & Sutton (1997)

or absent stromata, and shorter conidiophores. *Passalora caltaparum* (Chupp) U. Braun & Crous has longer conidia, superficial hyphae, and solitary shorter conidiophores. *Passalora markhamiae* (X.J. Liu & Y.L. Guo) U. Braun & Crous has smaller stromata and shorter conidiophores and conidia. *Passalora leprosa* (Speg.) U. Braun is easily distinguished by having much larger stromata (300–500 µm diam), longer conidiophores (40–150 µm), and shorter conidia (30–80 µm). Additionally, *P. catalpae* (Chupp) U. Braun & Crous has smaller (<50 µm) or no stromata, longer (≤ 125 µm) conidiophores that are geniculate and rarely septate, and conidia that are acicular-shaped, indistinctly septate, and longer (40–120 µm) than those of *P. macfadyenae*. *Passalora macfadyenae* causes severe necrosis and significant defoliation of its host in the field (Plate 5a) and appears to have good potential for use in classical biological control of *M. unguis-cati*.

Pseudocercospora unguis-cati (Speg.) U. Braun, Mycotaxon 51: 49. 1994. Plate 6

Lesions on living leaves, amphigenous, subcircular, well delimited, infected tissue initially dark brown surrounded by a pale brown halo, becoming grayish centrally with a narrow dark brown outer rim at the periphery, 3–35 mm diam., coalescing and leading to necrosis of extensive areas of leaves. Internal mycelium indistinct. External mycelium absent. Stromata absent or small and composed of only a few substomatal swollen cells, pale brown. Conidiophores hypophyllous emerging through stomata, solitary or forming loose fascicles of few conidiophores, straight to slightly curved, sometimes restricted to the conidiogenous cell, cylindrical, 11–50 × 2.5–5 µm, 0–2 septate, unbranched, light brown, smooth, with inconspicuous scars. Conidiogenous cells terminal, holoblastic, mostly cylindrical, 10–17.5 × 2.5–5, pale brown. Conidiogenous loci inconspicuous, flat, up to 2.5 µm, unthickened, not darkened. Conidia dry,

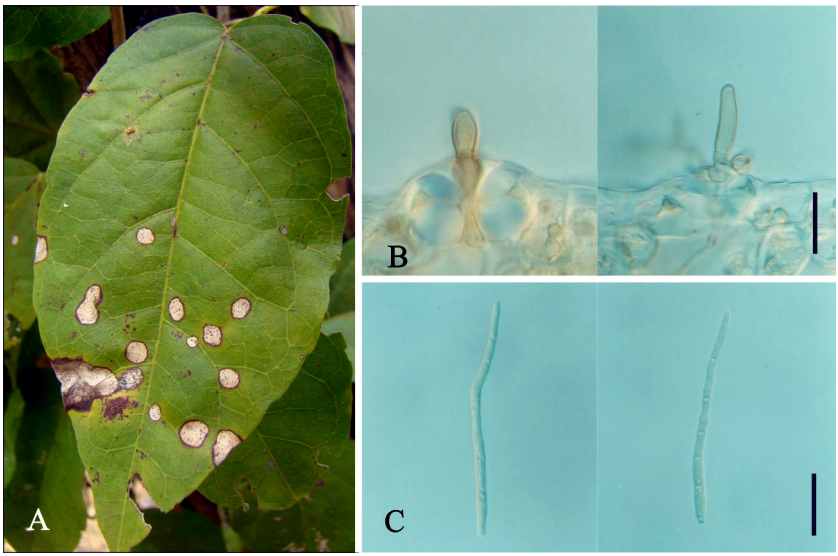

Plate 6. a. *Pseudocercospora unguis-cati* on *Macfadyena unguis-cati*: necrotic symptoms on infected leaves. b–c. *Pseudocercospora unguis-cati* (VIC 26575): b. conidiophores emerging through stoma; c. conidia. Bars = 10 µm.

solitary, acicular-obclavate, straight to slightly curved, 48–119 × 3.5–7 µm, base truncate, apex rounded, 0–6 septate, hilum unthickened, not darkened, pale brown, smooth.

Material examined: **BRAZIL**, **Minas Gerais**, on *M. unguis-cati*, 18 July 2004, OLPereira (VIC 26575); 18 May 2004, OLPereira (VIC 30479, 30481, 30482); 15 February 2005, OLPereira (VIC 30488); 2 February 2005, OLPereira (VIC 30489); 3 February 2005, OLPereira (VIC 30490, 30491); 25 July 2011, MSilva & OLPereira (VIC 31758). **Paraná**, on *M. unguis-cati*, 18 July 2004, OLPereira (VIC 26576). **São Paulo**, on *M. unguis-cati*, 26 July 2004, OLPereira (VIC 30486).

Comments: Only one cercosporoid species was previously known to occur on *Macfadyena* — *Pseudocercospora unguis-cati,* which was described from a specimen collected on *M. unguis-cati* in Argentina (Braun 1994). There are numerous *Pseudocercospora* species on hosts belonging to the *Bignoniaceae* (Crous & Braun 2003, Inácio & Dianese 2006). The survey in Brazil yielded two cercosporoid fungi that clearly belonged to the genera *Passalora* (see above) and *Pseudocercospora.* These were compared with the species described on *Bignoniaceae*. The *Pseudocercospora* specimen has morphological features that place it well within *P. unguis-cati* as described by Braun (1994). This is the first report of *P. unguis-cati,* originally described from Argentina, on *M. unguis-cati* in Brazil. *Pseudocercospora unguis-cati* causes necrotic spots on *M. unguis-cati*

(Plate 6a) that may be severe and should be further evaluated as a potential biocontrol agent.

Prospodium macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto, **sp. nov.**

MycoBank 561617 Plate 7–8

Differs from *Prospodium stizophylli* in larger (31.25–37.5 µm) teliospores, basket-like sori, and no mesospores and from *P. perornatum* in longer (47.5–55 µm) teliospores, larger (22.5–30 µm) urediniospores, and paraphyses within the sori.

Type: Brazil. Minas Gerais, on *Macfadyena unguis-cati*, 18 May 2004, OLPereira (VIC 30478, **holotype**).

Etymology: from the host genus name.

Lesions on living leaves, mainly on leaf veins but occasionally also widely distributed over the lamina, starting as chlorotic dots becoming pale brown centrally with well defined brown borders that later become dark brown to black and necrotic adaxially, appearing as black dots distributed within chlorotic areas, abaxially, of variable shape, 0.5–1.5 mm, leading to premature leaf fall. Internal mycelium intercellular 2.5–5 µm diam., branched, septate. Aecia unknown. Spermogonia epiphyllous, type 7, subepidermal, erumpent, in groups, conical, 16–38 × 6–19 µm, reddish brown. Uredinia and telia hypophyllous, in small scattered groups, basket-like sori, emerging through stomata, 152–221 µm diam., peripheral paraphyses 22.5–100 µm long, 5–12.5 µm wide, curved, cylindrical, somewhat inflated at base, tapering towards the acuminate apex, aseptate, chestnut brown, smooth; inner paraphyses, numerous, filiform to cylindrical, straight, 25–47.5 × 2.5–5 µm, aseptate, hyaline, smooth. Urediniospores globose to broadly ellipsoid, 30–37.5 × 22.5–30 µm, aseptate, wall bilaminate, the outer layer 2–2.5 µm thick, pale yellow, echinulate, inner wall up to 2.5 µm thick, yellow, germ pores, two, equatorial. Teliospores pedicellate, ellipsoid, 47.5–55 × 31.25–37.5 µm, rounded at both ends, one-septate, not constricted at the septum, wall 2.5–4.5 µm thick, chestnut brown, echinulate, two-pored, one apical pore in upper cell and one pore next to septum in the lower cell; pedicels attached basally or slightly obliquely, cylindrical, 50–62.5 × 5–7.5 µm, hyaline, persistent, with four or five conspicuous branched lateral appendages formed submedianly.

Additional material examined: **BRAZIL. Minas Gerais**, 25 Jul 2011, MSilva & OLPereira (VIC 31757).

Comments: Two rust fungi have previously been described in association with *M. unguis-cati*: *Uropyxis rickiana* Magnus [=*U. reticulata* Cummins] and *Prospodium stizophylli* H.S. Jacks. & Holw. (Cummins 1939; Carvalho Jr. et al. 2002, Hernández & Hennen 2003). *Uropyxis rickiana* is known only on *M. unguis-cati* from Argentina and Brazil (Hernández & Hennen 2003, Hennen et al. 2005) and *P. stizophylli* on *M. unguis-cati* and *Stizophyllum perforatum*

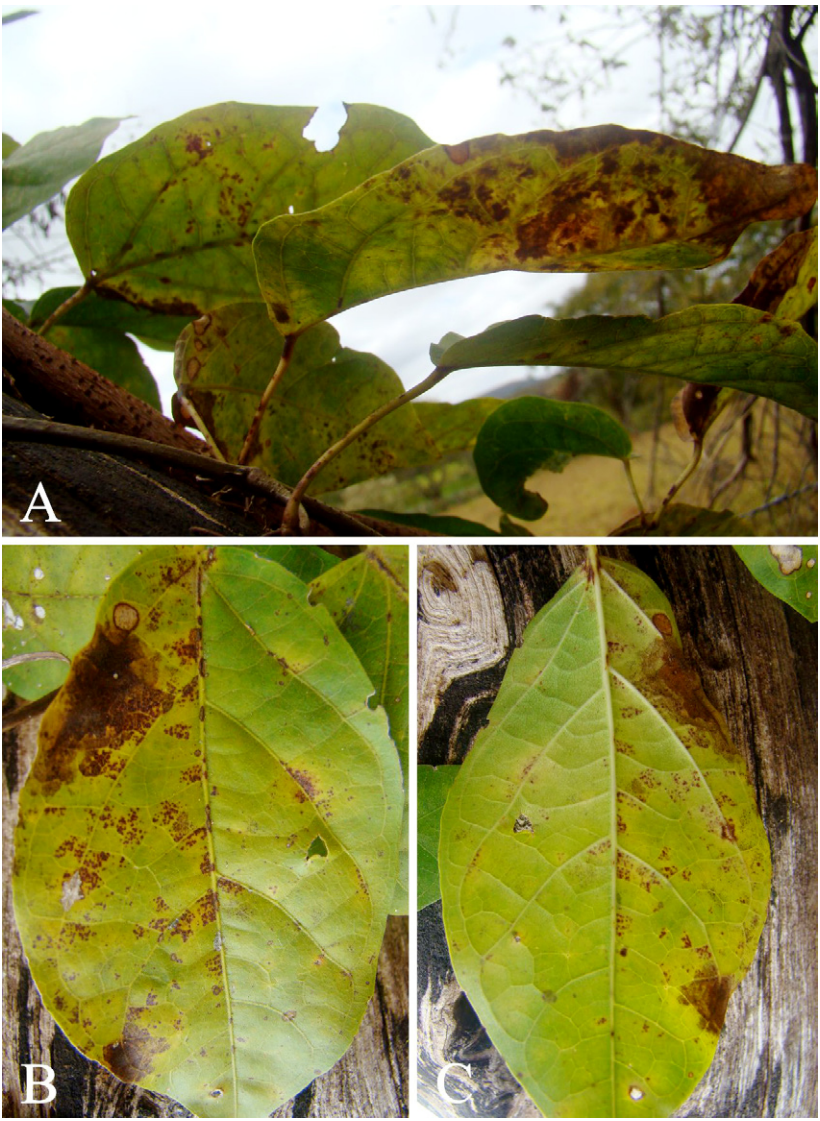

Plate 7. *Prospodium macfadyenae* on *Macfadyena unguis-cati*: A. Rust symptoms on infected plant; B. detail on adaxial leaf surface; C. detail on abaxial leaf surfaces.

(Cham.) Miers from Brazil (Carvalho Jr. et al. 2002). *Uropyxis* species differ from *Prospodium* species by having two pores on each teliospore cell instead of only one. The species collected during the present study clearly belongs to *Prospodium*. *Prospodium macfadyenae* differs from *P. stizophylli* by having

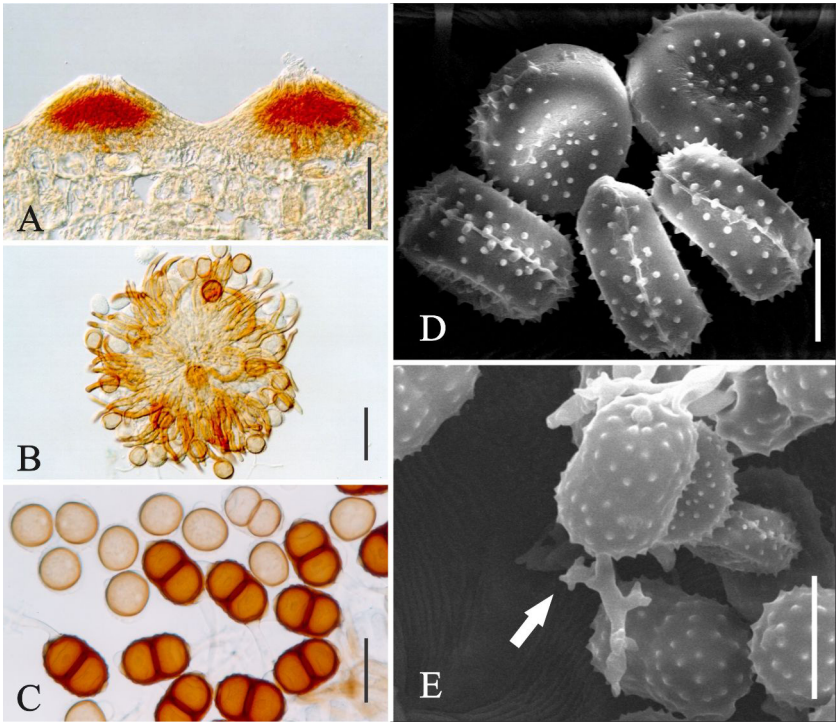

Plate 8. *Prospodium macfadyenae* (VIC 30478): A. type 7 spermogonium; B. basket-like sori (squash-mount); C. echinulate urediniospores intermixed with pedicellate 2-celled teliospores. Scanning electron micrograph (SEM) of spores: D. surface view of echinulate urediniospores; E. mature teliospores (note branched appendages on pedicels – arrowed). Bars: A = 10 µm; B = 50 µm; C = 30 µm; D = 10 µm; E = 20 µm.

longer, broader teliospores and the absence of mesospores. *Prospodium stizophylli* also does not have basket-like sori, does not produce uredinia, and has a pseudoreticulate ornamentation on its teliospores. *Prospodium macfadyenae* is morphologically most similar to *P. perornatum* Syd. (Cummins 1940, Hennen et al 2005), known only on *Tabebuia* spp. from Argentina and Mexico (Hernández & Hennen 2003), but differs from the latter by having larger urediniospores, longer teliospores that are not constricted at the septum, and the presence of paraphyses within the sori in addition to thick walled incurved peripheral paraphyses.

Prospodium macfadyenae causes significant damage to the foliage of *M. unguis-cati* (Plate 7), often leading to significant defoliation of its host, and belongs to a group of fungi that includes several highly successful classical biological control agents of weeds. Rust fungi generally have a high level of host-

specificity (and thus fit well into the safety requirements needed for classical introductions). Therefore, we regard the prospects for its use in biocontrol of *M. unguis-cati* as very good. Although *U. rickiana* has not been collected during the surveys in southern and southeastern Brazil, it is likely that this species also has potential as a biological control agent in warmer equatorial areas where *M. unguis-cati* occurs as an invasive plant. It has the same favorable features described for *P. macfadyenae* and causes a severe disease on its host producing conspicuous galls on stems as illustrated in Hernández & Hennen (2003).

Ideally, further investigations involving additional surveys and a more detailed evaluation of the potential biocontrol agents already uncovered are needed. These could yield additional candidates for use in classical biological control of *M. unguis-cati* and help pave the way towards introducing *Pseudocercospora unguis-cati*, *Passalora macfadyenae* and *Prospodium macfadyenae* into exotic situations where invasions by cat's claws are under way, contributing to the mitigation of the environmental damage caused by this important invasive weed.

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